Does helminth treatment reduce the risk of active tuberculosis in a cohort of children from a high tuberculosis risk population who have been vaccinated with BCG at birth?

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I dedicate this dissertation to my parents, James & Joan Finlay,

and my family – Mike, James & Jessica

DECLARATION

I, Lesley Workman hereby declare that the work on which this thesis is based is original (except where acknowledgements indicate otherwise) and that neither this work nor any part of it has been, is being, or is to be submitted for another degree at this or any other University.

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ABSTRACT

Background

Research in adults and older children has shown an association between *Mycobacterium tuberculosis* and helminth infection, with those infected with helminths at greater risk of tuberculosis. This association is believed to be on the basis that chronic helminth infection can result in a functional impairment of the immune response that is necessary to clear or control infection by *Mycobacterium tuberculosis* (Elias et al. 2001; Rook et al. 2006; Fincham 2001).

It is thus possible that the introduction of regular deworming programmes in a vulnerable population of children under the age of five years could assist their immune systems to ward off tuberculosis infection and reduce the risk of tuberculosis disease in such a population.

A randomised controlled trial to compare two methods of administering bacille Camlette-Guerin (BCG) vaccination to newborns from a high tuberculosis risk population provided an opportunity to test this hypothesis in a sub-study.

Objective

The objective of this study is to determine if young children in a high-risk tuberculosis population who have been vaccinated with BCG at birth and have been treated for helminth infection are at lower risk of tuberculosis disease than children who have been vaccinated with BCG at birth but not treated for helminth infection.

Method

A case control study nested within a cohort recruited for a separate randomised control trial to compare two methods of administering BCG vaccination was carried out. Children who presented to their local clinic or hospital with symptoms of tuberculosis or a history of exposure to tuberculosis were admitted to a case verification (CV) ward for investigation of tuberculosis.

Investigation of tuberculosis included a detailed history, including past helminth treatment, physical examination, tuberculin skin test, chest radiograph, gastric washing and induced sputum for culture of tuberculosis and clinical examination. A diagnostic algorithm was developed by specialist physicians and biostatisticians to classify the children into one of five tuberculosis categories.

A total of 510 children (median age 18.13 months) were included in the primary analysis of this case control study. Those defined as cases were the 328 classified as “definite or probable TB” and 182, classified as “not TB”, comprised the control group. Those classified as "possible TB" or "unlikely TB" were excluded.

A secondary analysis was performed that included the 337 children who had been classified as “unlikely TB” with the controls resulting in a total of 847 children (median age 18.37 months). The 328 children classified as “definite or probable TB” were defined as cases and the 519 classified as “unlikely or not TB” comprised the control group.

Univariate analysis was used to explore a possible relationship between tuberculosis and helminth treatment using all the variables in the sub-study (n=510 primary analysis; n=847 secondary analysis). For both the primary and secondary analysis a multivariate logistic regression model was built using a reduced sample that had a complete set of data for all the variables: primary analysis (n=435); secondary analysis (n=724). This final model was then fitted

on a more complete sample as the final variables selected had fewer missing data for the observations: primary analysis (n=493); secondary analysis (n=822).

Result

A total of 35.69% of the study sample in the primary analysis had been treated for helminth infection. The proportion of children who had been treated for helminth infection was similar in the cases and controls (35.98% and 35.16% respectively). Univariate logistic regression showed no association between tuberculosis and treatment for helminth infection: [odds ratio (OR) 1.04; 95% confidence interval (CI) 0.71 - 1.51].

Multivariate analysis adjusted for the effect of nutritional status, recorded as height for age z score (haz), number of occupants sharing the same dwelling as the child, gender and birth site showed a similar result: (OR 1.03; 95% CI 0.69 – 1.53). The OR is very close to 1 with a 95% CI that includes 1, which indicates that there is not a statistically significant association between tuberculosis and helminth treatment.

In the secondary analysis, a total of 38.61% of the study sample had been treated for helminth infection. In this analysis the proportion of children who had been treated for helminth infection showed a difference between the cases and controls (35.98% and 40.27% respectively). Univariate logistic regression showed a 17% relative reduction in tuberculosis odds but this was not a statistically significant result: (OR 0.83; 95% CI 0.63 – 1.11). Multivariate analysis adjusted for the effect of haz, number of children sharing the same dwelling as the child and gender, showed a similar result: (OR 0.85; 95% CI 0.63 – 1.15).

Conclusion

The primary analysis of this observational study does not support the hypothesis that helminth treatment reduces the risk of tuberculosis disease in young children in a high-risk tuberculosis population. Although the secondary analysis showed a 15% relative reduction in tuberculosis odds after adjusting for the effect of haz, number of occupants sharing the same dwelling as the child and gender, this was not a statistically significant result.

Final Conclusion

This study does not support the hypothesis that helminth treatment reduces the risk of tuberculosis disease in young children in a high-risk tuberculosis population.

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1. INTRODUCTION

Mycobacterium tuberculosis is the agent that causes tuberculosis, a disease that is estimated to infect about one third of the world's population (WHO, 2005). 29% of the global burden of tuberculosis is in sub-Saharan Africa where the incidence rate of smear positive tuberculosis in 2005 was 350 cases per 100 000 population (WHO, 2005).

Childhood tuberculosis contributes significantly to the global burden of this disease, with about one million children developing tuberculosis annually worldwide, which accounts for about 11% of all cases of tuberculosis (Marais et al. 2006; Fincham, 2001).

Childhood tuberculosis differs from adult tuberculosis by the way in which the immune system responds. Children under five years of age are more likely than adults to develop tuberculosis disease following infection and the time from infection to disease is likely to be shorter in children than in adults (Walls et al. 2004). This will have implications for the prevention of progression from infection to tuberculosis disease (Marais et al. 2006; Fincham, 2001).

Tuberculosis is contagious and spreads through the air. When a person with active pulmonary tuberculosis coughs, *Mycobacterium tuberculosis* is spread into the immediate environment in microdroplets which can remain in the air for prolonged periods. People in contact with an active case of pulmonary tuberculosis may inhale these micro droplets containing *Mycobacterium tuberculosis* (Elias et al. 2006; Grosset, 2003; Campbell et al. 2006). After infection with tuberculosis the infected person mounts a response to the pathogen which is so effective that on average 90% of humans with an immunocompetent system are able to control or clear the infection and avoid progression to tuberculosis disease (Hanekom et al. 2007; Elias el al. 2001; Grosset, 2003).

The variation in the ability of the human body to handle tuberculosis has been explored (Elias et al. 2001). Various suggestions regarding the difference in protection against tuberculosis disease have included exposure to environmental mycobacteria (atypical mycobacteria), nutritional differences, genetic or physiological differences and perturbation of the immune system by chronic infectious diseases including helminth infection.

Specifically, chronic helminth infection can result in functional impairment of the immune response that is necessary to clear or control infection by *Mycobacterium tuberculosis.* Protection against tuberculosis is associated with a predominant T helper lymphocyte Type 1 (Th1) cell mediated immune response to mycobacterial antigens, characterised by interferon-gamma release, while susceptibility to tuberculosis is associated with a reduced Th1 response. Helminth infection results in a depressed Th1 cell mediated response, and Th2 polarisation, with or without an elevated T regulatory lymphocyte (Treg) response, which results in a reduction of interferon-gamma production necessary for protection against *Mycobacterium tuberculosis* (Elias et al. 2001; Rook et al. 2006; Fincham 2001).

Helminth infection could thus have a marked effect on the human response to *Mycobacterium tuberculosis* and facilitate the spread of this disease (Bundy et al. 2000; Elliot et al. 2007; The Lancet Editorial, 2004).

Communities with a high prevalence of helminth infection are often those where the incidence of tuberculosis is high – typically those where the living conditions are poor and where over-crowding, poverty and malnutrition are common (Elias el al. 2001; Campbell et al. 2006). It is estimated that more than two billion people worldwide have helminth infections. In endemic areas, children could be infected as soon as they are able to crawl. (Elias et al. 2001). In a study conducted in the Eastern Cape and KwaZulu-Natal, about 20% of children aged less than one year were infected with helminths (Smuts et al. 2004).

There is convincing evidence that helminth control in the Western Cape is neglected (Bentwitch et al. 1999). Established benefits of community control of helminth infection include enhancing development in children, reduction in anaemia and reduction in malnutrition (Alderman et al. 2006).

In addition to enhancing the nutritional status, growth and intellectual development of children (WHO, 2005), there is accumulating evidence that prevention of helminthiasis by mass deworming could improve the immune response to tuberculosis infection and thus potentially decrease susceptibility to tuberculosis and slow progression to disease once infected (Adams et al. 2005; Marais et al. 2006; Fincham, 2001; Bundy et al. 2000; The Lancet Editorial, 2004).

It is thus possible that introduction of regular, six-monthly deworming of children under the age of five years and the inclusion of mass deworming in immunisation campaigns could result in a reduction of helminth infections in this age group, and result further in a reduction in tuberculosis disease.

Between 26th March 2001 and 31st July 2006, a randomised controlled trial was conducted by the South African Tuberculosis Vaccine Initiative which compared two methods of BCG vaccination administration in preventing tuberculosis in very young children.

The primary objective of the trial was to compare the efficacy of the percutaneous to that of the intradermal route of administering BCG in preventing tuberculosis during the first two years of life. The primary endpoint of the trial was the incidence rate of tuberculosis during the first two years of life.

The study was based in the Boland Overberg region of the Western Cape in the Republic of South Africa, centred at Worcester, where the burden of tuberculosis is high. Smear positive detection rate of tuberculosis in this area

was 602/100 000 in 2004 (Nicol et al. 2008). The population has a relatively low HIV sero-prevalence for South Africa: between 5.7 and 6.2% of pregnant women attending public health service antenatal clinics are HIV sero-positive (Hawkridge et al. 2008). The estimated incidence rate of tuberculosis disease in children aged younger than five years is about 2 500/100 000 per annum, which is very high (Hawkridge et al. 2008).

In addition to the trial collecting information on tuberculosis, demographics and socio-economic status, information was collected on previous helminth treatment. This study thus provided an opportunity to test the hypothesis that helminth treatment reduces the risk of tuberculosis disease in young children from a high tuberculosis risk population.

1.1 Rationale

It is important to establish whether children who are treated for helminth infection are protected against tuberculosis.

As tuberculosis is a common disease, a reduction in tuberculosis cases due to introduction of mass deworming programmes in children younger than five years of age could significantly decrease the burden of tuberculosis disease in this age group. This would be strong motivation to introduce regular deworming programmes to include preschool children and include mass deworming treatments in immunisation campaigns.

1.2 Objective

The objective is to determine the association between past helminth treatment and the risk of tuberculosis disease in young children from a high-risk tuberculosis population.

1.3 Study hypothesis

In a high-risk tuberculosis population, young children who have been vaccinated with BCG at birth and have been treated for helminth infection will have lower odds of tuberculosis disease than children who have been vaccinated with BCG at birth but not treated for helminth infection.

2. LITERATURE REVIEW

2.1 Objective of literature review

The objective of this literature review was to assess existing evidence of the effect of chronic helminth infection on the immune system, as well as evidence of a possible association between tuberculosis and helminth infection or helminth treatment, to critically appraise this evidence and to summarise the outcomes of this research.

2.2 Search strategy

Using the key words “helminth infection” or “worm infestation” or “helminth treatment” and “tuberculosis” or "TB", the following resources were searched to identify existing evidence of an association between helminth infection and tuberculosis:

Medline / Pubmed

EMBASE

Cochrane Library

Google

In addition, specialist journals on infectious disease were scanned manually and physicians were asked for guidance by means of personal communication.

2.3 Summary of relevant research

Helminth infections are common in large parts of the world and it is estimated that more than a quarter of the world’s population are infected. This burden is greatest in developing countries especially Africa, South-East Asia and South

America where the burden of other infectious diseases, including tuberculosis, is also high (Borkow et al. 2000; Bundy et al. 2000).

During a period of 15 years, about 60 000 Ethiopians emigrated from Ethiopia to Israel. More than 80% of these immigrants were infected with a least one helminthic parasite.

The immigration of these Ethiopians allowed researchers the opportunity to investigate the effect of chronic immune activation caused by helminth infection on immune responses and the ability of the immune system to cope with infections (Borkow et al. 2000).

Three groups were identified and included in the study. The first group comprised recently immigrated Ethiopians with helminth infection, before deworming. The second group comprised Ethiopians that had been living in Israel for at least three years, had been treated for helminth infection and were at the time of the study free of helminth infection. The third group comprised healthy Israeli-born helminth free individuals. The immune profiles of the three groups were analysed and compared (Borkow et al. 2000).

The study showed a clear dysregulation of several immune parameters in those Ethiopians recently immigrated to Israel compared to the group that had been born in Israel. The differences between the group who had immigrated to Israel at least three years before the study and the group who had been born in Israel were not as great, suggesting that the immune profiles of the Ethiopians gradually returned to normal after immigration to Israel (Borkow et al. 2000).

The researchers concluded that although the improvement in health care and nutrition would have contributed to an improvement in the immune system, eradication of helminth infection was the most important factor in this improvement. (Borkow et al. 2000).

The health benefits of deworming preschool children have been studied in eastern Uganda. A randomised controlled trial was used to investigate the effect of including an anthelmintic in a new health programme for children. The study population was cluster randomised by parish.

Out of the 48 parishes included in the new health programme, 24 parishes offered children aged between one and seven years anthelmintic treatment in addition to standard services on child health days, over a three-year period (Alderman et al. 2006).

The main outcome of the study was weight gain. The results of the study showed about a 10% increase in weight gain in children from the parishes that had been randomised to offer an additional anthelmintic treatment to standard services: [166 g per child per year; (95% CI 16–316)] above expected weight gain when anthelminic treatment was given bi-annually. The weight gain was 5% if the treatment was given annually (Alderman et al. 2006).

The researchers concluded that regular deworming of preschool children, as part of regularly scheduled health services, was practical and resulted in weight gain (Alderman et al. 2006).

The question has been raised in the research community of additional health benefits of deworming young children including possible protection against tuberculosis disease (Bundy et al. 2000; Tristao-Sa et al. 2002; Elias et al. 2006).

Available epidemiological data regarding associations between helminth infection and tuberculosis are largely observational. Two previous studies have shown a significantly higher prevalence of helminth infection in patients with pulmonary tuberculosis compared to control groups as shown in Table 1 (Tristao-Sa et al. 2002; Elias et al. 2006).

Table 1: Summary of previous studies

Author	**Study population**	**Study design**	**Potential confounders** (not adjusted for)	**Effect**
Tristao-Sa (2002)	Adults (hospitalised)	Matched case control	- nutritional status - HIV status	OR 5.19 (2.33 – 11.69)
Elias (2006)	Adults & older children (students)	Matched case control		OR 4.2 (2.7 – 8.7)

Between 1997 and 1999, researchers conducted a case control study in hospitalised patients in Brazil. Stool samples were examined for intestinal nematodes in 57 cases of pulmonary tuberculosis and 86 randomly selected stool examinations from patients with other diseases. The control group individuals were matched to the cases by age, gender and location. The results showed that prevalence of helminths was five times higher in the patients with pulmonary tuberculosis than in the control group: (OR 5.19; 95% CI 2.33 - 11.69) (Tristao-Sa et al. 2002). Although the cases and controls were matched by age, gender and neighbourhood, these results could have been confounded by HIV status which was not evident in the article. As the patients were from the same community it is unlikely that socio-economic status was a confounder.

This result was confirmed in a study done by Elias between October 1999 and 2002 in Ethiopia, where both tuberculosis and helminth infections are endemic (Elias et al. 2006). The study design was case control: 230 cases with clinical features of tuberculosis and smear positive sputum were compared to 510 controls who were selected from the same household as the case, had been resident in the same house for at least 12 months and were found to be free of any signs and symptoms of tuberculosis on normal physical examination.

The researchers found a strong association (OR 4.2; 95% CI 2.7 – 5.9) between tuberculosis and helminth infection and concluded that intestinal

helminth infection together with HIV infection, poverty and poor living conditions may be an important risk factor for tuberculosis (Elias et al. 2006). Age, gender and HIV status were treated as potential confounding variables. The researchers adjusted for their possible effect by including them in the logistic regression model. As the controls were selected from the same household, it is unlikely that confounding by nutritional status would have influenced these results.

These results support the hypothesis that the immune perturbation induced by helminth infection may lessen the host's response to *Mycobacterium tuberculosis*, thus facilitating infection and progression to tuberculosis disease. If this hypothesis holds, eradication of helminth infections could have a significant impact on control of tuberculosis in the developing world (Tristao-Sa et al. 2002; Bentwich et al. 1999).

However, although the studies mentioned above found strong associations between helminth infection and tuberculosis disease in adults and older children (age range 11 to 79 years), with ORs of 5.19 and 4.2 respectively, little is know about the effect of helminth treatment on the incidence of tuberculosis disease in children or adults.

The first trial to examine the effects of helminths and deworming on tuberculosis among young children is currently being conducted in Uganda. The study is a randomised controlled trial which will allow the role of helminths and the benefits of deworming to be determined in relation to infectious diseases including tuberculosis, and the effect on anaemia, growth and intellectual development is to be assessed. The trial has three randomised, double blinded, placebo controlled interventions. Pregnant mothers are randomised to receive either albendazole or placebo and praziquantel or placebo. At the age of 15 months their children are randomised to receive either albendazole or placebo given every three months; to continue until the child is five (Elliot et al. 2007).

In the absence of results from randomised trials, observational data may help to strengthen the case for an additional benefit of deworming.

3. METHODS

This study was conducted in the Boland Overberg region located in the Western Cape Province of South Africa, in the municipal districts of Breede River Winelands, Breede Valley and Witzenberg. The economy is based on agriculture and tourism resulting in the population being small and dispersed. The regional centre is the town of Worcester.

3.1 Study design

A case control study nested within a cohort recruited for a separate randomised control trial to compare two methods of administering BCG vaccination as described earlier was carried out.

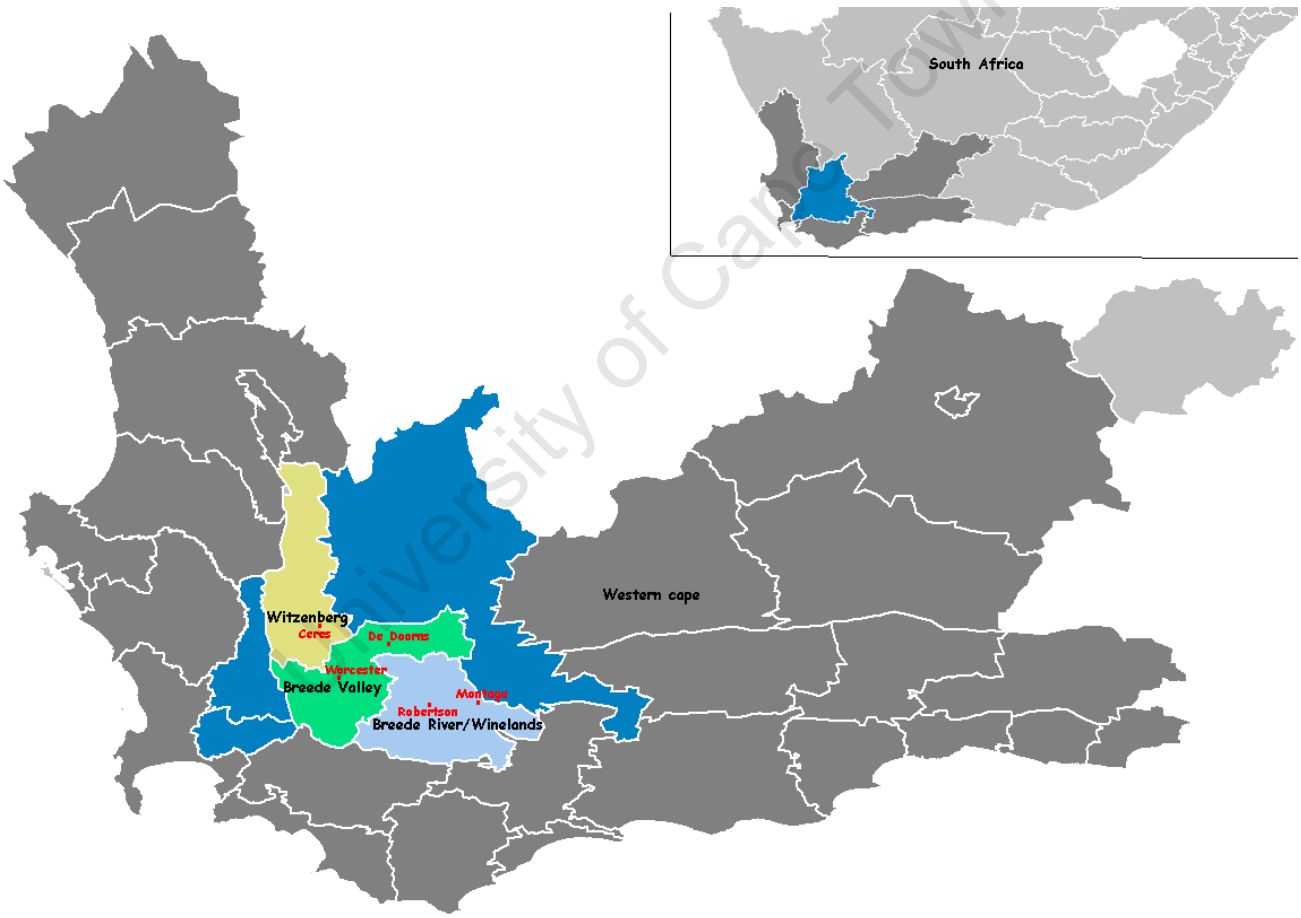
3.2 Study site and study population

The study was carried out in a high tuberculosis burden population. The smear positive detection rate of tuberculosis in this area was 602/100 000 in 2004 (Nicol et al. 2008). The population has a relatively low HIV sero-prevalence for South Africa: between 5.7 and 6.2% of pregnant women attending public health service antenatal clinics (Hawkridge et al. 2008). The estimated incidence rate of tuberculosis disease in children aged less than five years is about 2 500/100 000 per annum, which is very high (Hawkridge et al. 2008).

The comparison of the two methods of administering BCG was needed as the intradermal method of administration of BCG is almost universally accepted over percutaneous administration without direct evidence from a clinical trial. The reasoning behind this was that although it was easier to administer percutaneous BCG, the intradermal method provided for a more accurate dose.

In 1999 the South African Department of Health decided to fall in line with World Health Organization (WHO) recommendations to change the method of BCG vaccination from percutaneous to intradermal. However, it was the view that additional research on the methods of administering BCG vaccination should be carried out. This trial provided the opportunity to carry out the sub-study described in this dissertation.

Figure 1: Map of the Western Cape showing the Boland Overberg region



3.3 Sample size and randomisation of the cohort study

The study cohort comprised 11 680 neonates, vaccinated with BCG within 24 hours of birth, whose mother had signed informed consent and were enrolled in the study. Children excluded were those who were not eligible to receive routine BCG vaccination within 24 hours of birth, were not from the study area or were not born at one of five public maternity health units.

The randomisation sequence was generated by the study statistician in Microsoft Excel. The randomisation was by week and balanced in eight-week blocks of four intradermal and four percutaneous in one eight-week cycle.

The children were enrolled by midwives and study counsellors who were aware of the week's vaccine allocation. Allocation concealment and masking did not take place.

3.4 Surveillance for tuberculosis and referral to the case verification ward

The cohort was followed up for at least two years after BCG vaccination. Active surveillance for tuberculosis disease was used for the first three months. A total of 4 851 children were screened for incident illness that could have been tuberculosis when they attended a local health facility for routine vaccination visits at six, 10 and 14 weeks of age. Any children with a history of exposure to tuberculosis or those who presented with symptoms suggestive of tuberculosis were admitted to a specialised tuberculosis case verification ward at Brewelskloof Hospital, Worcester. Passive surveillance was based on scanning hospital admission lists, tuberculosis registers and mortality records for illness suggestive of tuberculosis or death. All surveillance events that were reported that could have been tuberculosis were investigated by a member of the study staff.

The following symptoms were considered suggestive of tuberculosis: cough, fever, night sweats, loss of weight for two weeks or more, or any episode of haemoptysis.

3.5 Investigation of tuberculosis

A ward at the regional tuberculosis referral hospital in Worcester was equipped to carry out procedures required for the diagnosis of tuberculosis in children. Investigation for tuberculosis included a detailed history, physical examination, tuberculin skin test, chest radiograph and culture of sputum obtained through early morning induced sputum and gastric lavage. All mothers were asked to consent to an HIV rapid test if the child was younger than 18 months and HIV polymerase chain reaction if the child was older than 18 months. Both Mantoux and Tine tuberculin skin tests were administered. A Mantoux reaction of greater than or equal to 15 mm was considered positive in HIV negative children and a Mantoux reaction of greater than or equal to 4 mm was considered positive in HIV positive children. A Tine reaction of greater than grade 2 was considered positive in all children.

A panel of three expert chest x-ray reviewers classified the chest radiograph into one of five TB classifications: “Highly likely TB”, “likely TB”, “suspicious of TB “, “abnormal but not TB” and “normal”.

3.6 Tuberculosis diagnostic algorithm and choice of cases and controls

A TB diagnostic algorithm was developed by specialist physicians and biostatisticians to classify the study population into one of five tuberculosis categories: definite TB, probable TB, possible TB, unlikely TB and not TB (Table 2) (Hawkridge et al. 2008).

Table 2: Definition of tuberculosis classification

TB Classification	Defined as
Definite	microbiological confirmation of *Mycobacterium tuberculosis*.
Probable	chest radiograph compatible with TB at least one additional feature of TB[1].
Possible	chest radiograph compatible with TB with no additional features of TB[1];
	or
	chest radiograph not suggestive of TB and diagnosed as TB by the treating physician;
	or
	chest radiograph not suggestive of TB and two or more additional features.
Unlikely	negative sputum microbiology, chest radiograph not suggestive of TB, at least one additional feature of TB[1];
	or
	diagnosed as TB by the treating physician without any other criteria being met.
Not	negative sputum microbiology, chest radiograph not suggestive of TB, no additional features of TB[1], not diagnosed as TB by the treating physician.

[1] Additional features:

cough for at least two weeks

failure to thrive

recent loss of weight

smear positive for acid fast bacilli (AFB)

Mantoux ≥ 15mm

For the purpose of this case control study two types of control group were defined. For the primary analysis, those admitted to the hospital for investigation and classified as definite or probable TB were defined as cases, and those classified as not TB comprised the control group.

In the secondary analysis, those classified as unlikely TB were included in the control group.

The two-year follow up of the 11 680 study subjects ended in 2006. In this period 1 445 children had been admitted to the case verification ward for investigation of tuberculosis. Some of the children were admitted more than once, resulting in 1 867 admissions to the case verification ward.

Table 3: Summary of admissions to the case verification ward by tuberculosis classification.

(n=1 867)

TB Classification	n (%)
Definite TB	193 (10.3)
Probable TB	280 (15.0)
Possible TB	438 (23.5)
Unlikely TB	599 (32.1)
Not TB	357 (19.1)

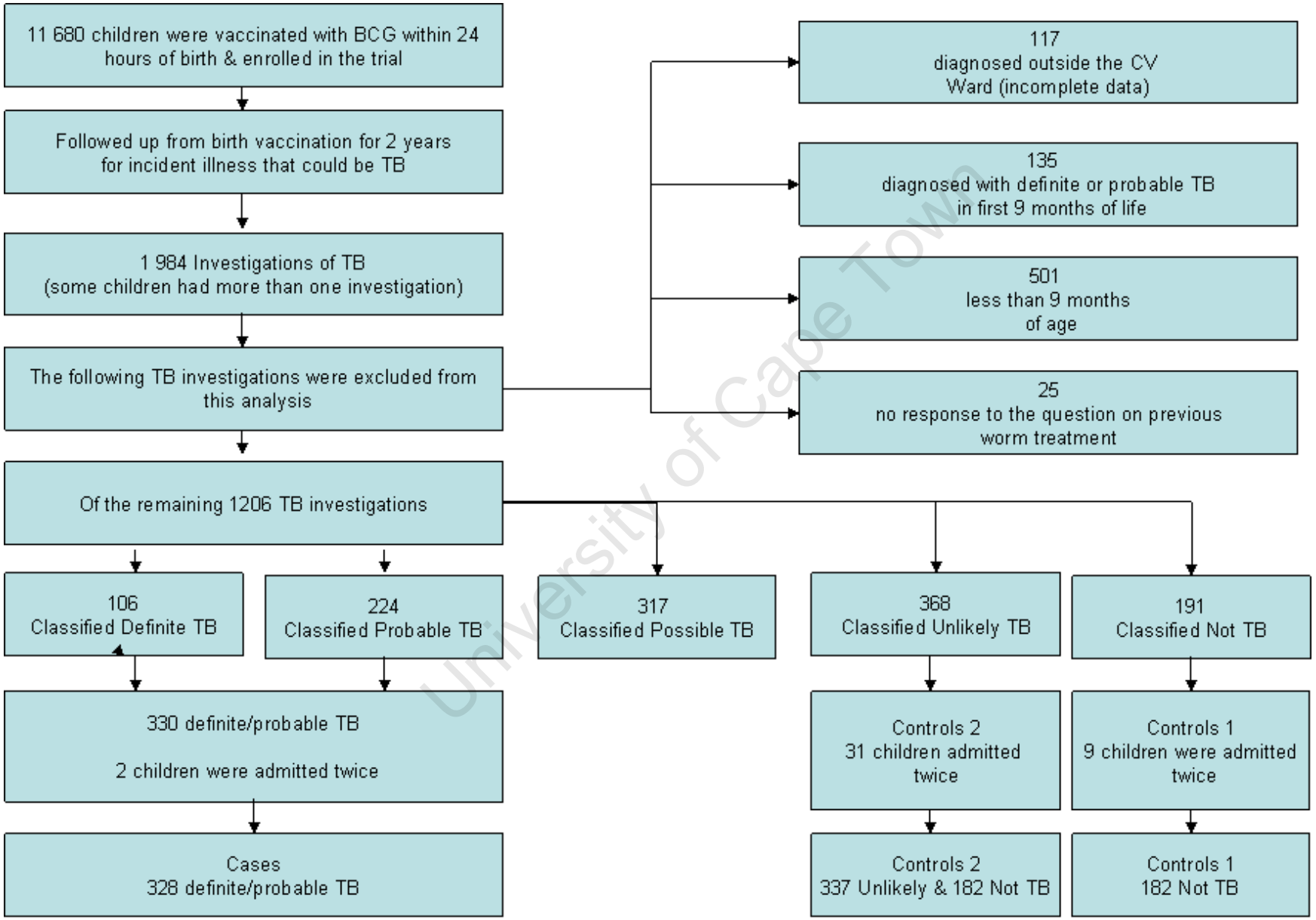
At each admission to the case verification ward a full tuberculosis evaluation was performed. For this study only one admission per child was used in the analysis. A total of 11 children who were analysed in this case control study were admitted to the CV ward more than once. In this instance, the admission that was selected for the analysis is described in Table 4.

Table 4: Selection of admission

TB classification	Admission selected
If both of the admissions were classified as definite or probable TB	The first admission was used in the analysis
If both of the admissions were classified as not or unlikely TB	The first admission was used in this analysis
If one admission resulted in a definite or probable TB classification and the other an unlikely or not TB classification	The admission classified as definite or probable was used in this analysis

Figure 2 summarises the cohort enrolled for the trial, the number of children assessed for possible tuberculosis, the exclusion criteria for this case control sub-study and finally those selected as cases and controls for this sub-study.

Figure 2: Flow diagram of patient numbers and case control study population selection



3.7 Exclusions

The following tuberculosis investigations were excluded from this analysis:

- 117 tuberculosis cases diagnosed outside the case verification ward. These children had been diagnosed as tuberculosis cases and started on tuberculosis treatment by the public health service before the study staff were able to admit them to the case verification ward for investigation of tuberculosis. These children were not included in this analysis as the diagnosis of tuberculosis had not been made using the diagnostic algorithm, it was also not known if any of the children had been treated for helminth infection.
- 135 tuberculosis cases diagnosed as definite or probable tuberculosis in the first nine months after birth. These children were diagnosed before they had much chance of helminth treatment.
- 501 tuberculosis investigations in children younger than 9 months. Very few children were treated for helminth infestation before the age of nine months.
- 25 whose parents/guardians had no response to the question “was your child treated for worms?”

A summary of the remaining 1 206 tuberculosis investigations are shown in Table 5.

Table 5: Summary of tuberculosis classification after general exclusions (n = 1 206)

TB Classification	n (%)
Definite TB	106 (8.8)
Probable TB	224 (18.6)
Possible TB	317 (26.3)
Unlikely TB	368 (30.5)
Not TB	191 (15.8)
Total	1 206 (100.0)

Of the 330 cases (106 definite TB, 224 probable TB), two children had been admitted twice, leaving 328 cases for this analysis. Of the 191 controls (not TB) for the primary analysis, 9 children had been admitted twice, leaving 182 controls for this analysis.

In the secondary analysis, of the additional controls from those classified as unlikely TB (368), 31 children had been admitted twice, leaving 337 unlikely TB. The total control group for the secondary analysis was thus 519.

The 317 children who were classified as possible TB were not used in this analysis on the grounds that the children in this group were not suitable for a case diagnosis of tuberculosis but could not be used as controls as they had some features that could have been tuberculosis.

Table 6: Final case and control selection
(primary analysis)

TB Classification	Number	Allocation
Definite TB	106	case
Probable TB	222	case
Not TB	182	control

The final data set for the primary analysis included 328 cases and 182 controls as shown in Table 6.

Table 7: Final case and control selection
(secondary analysis)

TB Classification	Number	Allocation
Definite TB	106	case
Probable TB	222	case
Unlikely TB	337	control
Not TB	182	control

The final data set for the secondary analysis included 328 cases and 519 controls as shown in Table 7.

The secondary analysis was performed to establish if the result of the primary analysis held true when those classified as “unlikely TB” were included in the control group as well as to increase the power of the study.

As one of the reasons that a child might have been treated for helminth infection by the public health service was for loss of weight or failure to thrive unrelated to tuberculosis, and these symptoms had been excluded by the TB diagnostic algorithm in the “not TB” group, it was decided to include this group to determine if the result of the primary analysis was different given that more of the control group might have been treated for helminth infection.

3.8 Power of the study

The power of the study was calculated to detect differential proportions treated for helminth infection among cases and controls as shown in Tables 8 and 9 given the fixed sample size.

Table 8: Estimated power of the study
(primary analysis)

Proportion of cases treated for helminth infection	Proportion of controls treated for helminth infection	OR	95% CI	Power
15%	25%	0.53	0.34 - 0.84	74%
18%	30%	0.52	0.34 – 0.79	84%
25%	40%	0.51	0.35 – 0.75	92%

Given these assumed proportions of cases and controls treated for helminth infection, and given 328 cases and 182 controls, the primary analysis was powered in excess of 70% to prove a 50% relative difference statistically significant at the 5% level, between helminth treatment and tuberculosis, as shown in Table 8.

Table 9: Estimated power of the study
(secondary analysis)

Proportion of cases treated for helminth infection	Proportion of controls treated for helminth infection	OR	95% CI	Power
15%	25%	0.49	0.34 - 0.71	93%
18%	30%	0.52	0.36 – 0.73	97%
25%	40%	0.50	0.37 – 0.69	99%

By including those classified as “unlikely TB” in the control group, the power of the study was increased to greater than 90% to prove a 50% relative difference statistically significant at the 5% level, between helminth treatment and tuberculosis given these assumed proportions, and given 328 cases and 519 controls as shown in Table 9.

3.9 Measurement

3.9.1 Demographic patient data

The following data were recorded at enrolment and analysed in this case control study: child's date of birth, gender, birth weight, birth length, gestational age, birth site, mother's date of birth and ethnicity.

3.9.2 Tuberculosis investigation data

The following data were recorded at the time of admission to the case verification ward and analysed in this case control study: date of admission, number of referrals to the case verification ward, height at admission, type of housing (formal or informal), type of community (urban or farm), number of occupants sharing the same dwelling as the child and previous treatment for a helminth infection.

3.9.3 Tuberculosis diagnostic algorithm data

The following data were recorded at admission to the case verification ward and programmed by the diagnostic algorithm to classify each admission into one of five tuberculosis categories: smear and culture sputum microscopy, chest radiograph, Mantoux and Tine tuberculin skin test, clinical symptoms of cough for more than two weeks, failure to thrive, recent loss of weight, HIV status and a diagnosis of tuberculosis by the treating physician.

The variables that have been used for this analysis, the codes ascribed and the method of determining derived variables are summarised in Table 10.

Table 10: Variables extracted from the database of the main study.

Variable name	Comment
Mother's age (years)	Mother's age at birth calculated as [Child'sDateOfbirth]-[Mother'sDateOfbirth]
Child's age at admission (months)	Age at admission calculated as [DateOfAdmission]-[DateOfBirth]
Gender	Gender coded as: male=1; female = 0
Ethnicity	coded as: black = 1; coloured = 2; asian = 3; white = 4
Birth weight (kg)	
Low birth weight	A birth weight <2.5 kg coded as low birth weight
Gestation (weeks)	Gestation <37 weeks coded as an early birth
Type of community	Farm or urban
Type of housing	Formal or informal
Number of people living in the same house	A measure of socio-economic status
Birth site	Birth sites coded as: Worcester = 1; Ceres = 2; Robertson = 3; Montagu = 4
TB diagnosis	Coded as: Definite/probable TB = 1; not TB = 0 For the secondary analysis, unlikely TB = 0,not TB=0
Treatment for helminth infection	Coded as: yes = 1; no = 0
Height (cm)	Height in cm at admission was used to calculate the height for age z score
HIV status	Coded as: positive = 1; negative = 0

A random 10% quality check was completed on the data. If the child had been treated for helminth infection by the local health service this treatment would have been recorded on the Road to Health Card (RTHC). The RTHC is issued to the mother after the birth of her child: it is a tool that is used by nurses and

doctors to monitor the child's development in the first five years of life. It is also used to record immunisations and additional treatments including helminth treatment. The RTHC was also used as an additional source of data if data were missing from the study case report form (CRF). Data that were not recorded or measurements that were not taken are summarised in Tables 11 and 12.

Table 11: Missing variables by group

Variable	Total	Case	Control
Ethnicity	22	11 (3.4%)	11 (6.0%)
Mother's date of birth	12	7 (2.1%)	5 (2.7%)
HIV status	29	21 (6.4%)	8 (4.4%)
haz	8	5 (1.5%)	3 (1.6%)
Number of occupants	9	2 (0.6%)	7 (3.8%)

Table 12: Missing variables by helminth treatment

Variable	Total	Helminth treatment (Rx)	No helminth Rx
Ethnicity	22	6 (3.3%)	16 (4.9%)
Mother's date of birth	12	3 (1.6%)	9 (2.7%)
HIV status	29	15 (8.2%)	14 (4.3%)
haz	8	1 (0.5%)	7 (2.1%)
Number of occupants	9	4 (2.2%)	5 (1.5%)

The missing variables appear to be randomly scattered thoughout the data-set which will reduce any bias that could be introduced by reducing the sample to include only variables that do not have missing values in the model selection for multivariate analysis.

3.10 Ethical considerations

Written, informed consent was obtained from parents or guardians of all study participants at initial trial enrolment such that they understood that the child would receive standard BCG vaccination administered either percutaneously or intradermally. The parent or guardian was informed that if the child suffered any effects as a direct result of the BCG vaccination, medical care would be provided by the public health services. No other compensation was offered.

Written, informed consent was obtained from the parents or guardians of all study participants who were admitted to the case verification ward for investigation of tuberculosis. This consent included information on HIV and permission to take blood for an HIV test. Counselling was offered before and after HIV testing. On discharge the parents or guardians were compensated with a small hamper of baby products or a small toy to the value of R50.00.

The study was approved by the Research Ethics Committee of the University of Cape Town. (REC Ref 271/2000).

3.11 Statistical analysis

The data were analysed using STATA 8 statistical software (STATA Corporation, College Station, TX USA) and EpiInfo version 6 [Database and statistical software for public health professionals, Centres for Disease Control and Prevention (CDC)].

Exploratory data analysis of categorical and continuous variables included frequency tables, and histograms of continuous variables to determine distribution. Normally distributed continuous data were summarised by mean and 95% CI, and non-normally distributed continuous data by median and

interquartile range. Categorical data were summarised as proportions with 95% CIs.

Nutritional status of the children was calculated as height for age z score (haz) using date of birth, date of measurement, gender and height at admission. The haz score was calculated using the CDC/WHO 1998 reference (WHO, 1997). Height for age z score (haz) is a tool that can be used to assess a child's growth and general nutritional status using a standardised age and gender specific growth reference. The z score is expressed as standard deviations above or below the reference mean. The reference has an expected mean z score of 0 and a standard deviation of 1.0 (WHO, 2007). A haz below -2 describes stunting, which implies long term malnutrition and poor health (WHO, 2007).

Univariate and multivariate logistic regression analysis were used to explore the association between helminth treatment and tuberculosis.

Potential confounding variables are variables that are related to the outcome, which in this study is tuberculosis disease, among those not exposed to helminth treatment and, in addition, are related to the exposure variable, which in this study is helminth treatment.

All co-variables were first tested for potential confounding using the following method:
Univariate analysis was used to investigate:

1. the relationship of potential confounders with tuberculosis disease amongst the subset of children not treated for helminth infection;

   and

2. the relationship of potential confounders with exposure to treatment for helminth infection.

If variables were found to be associated with both these outcomes, they were considered potential confounders and added to the model that just contained helminth treatment to investigate the association between helminth treatment and tuberculosis disease after adjustment for potential confounding variables.

Each co-variable was added separately to the best model of confounders – nested models were compared using the likelihood ratio chi-square statistic (lrtest) and both nested and non-nested models were compared using the Akaike's information criterion (AIC). Model building continued until adding co-variables did not improve the model.

Model checking procedures were accomplished by calculating residuals. A plot of standardised Pearson residuals versus the linear component of the model was used to check both the functional form of the model and to identify outlying observations.

Dummy variables were created for birth sites Ceres, Robertson and Montagu, using the birth site of Worcester as the reference category.

The secondary analysis was performed as above on the data that included those classified as "unlikely TB" in the control group.

4. RESULTS

4.1 Quality of information

The data quality was believed to be of a high standard as the study population were admitted to the case verification ward where trained clinical research nurses took a detailed history and performed the necessary tests.

To validate the accuracy of previous treatment for helminth infection, a 10% sample of CRFs were compared to the child's Road to Health Card (RTHC) where a record of helminth treatment was recorded by staff at the local health facility. No discrepancies in the 10% sample check that was discussed in the measurement section, between the data recorded on the CRF and the RTHC, were found. It is possible that the interviewer used the RTHC as a source of previous helminth treatment in addition to the mother's recall.

4.2 Baseline characteristics of the study population

The baseline analysis included 510 children as shown in Table 13.

The mean age of the mother at the child's birth was 25.71 years (range: 14.18 – 43.64). 48 (9.4%) of the mothers were younger than 18 years at the child's birth.

23.53% of the mother's reported that they lived on a farm and the number of people sharing the same dwelling with the child had a median of 5 (range: 1 - 20).

9.41% reported that they were living in informal housing, which included any type of shack (zinc, wood or iron) or "wendy house" that was free standing and did not have water or toilet facilities.

The median gestational age at the child's birth was 39 weeks (range: 29 – 44). An early birth was defined as gestational age of less than 37 weeks; 53 (22.16%) mothers gave birth before 37 weeks of pregnancy.

The majority of the children (52.55%) were born in Worcester at either the Eben Donges Hospital or the Maria Pieterse Maternity Obstetric Unit. The other study birthing sites were Ceres Hospital (18.24%), Robertson Hospital (17.25%) and Montagu Hospital (11.9696%).

The majority of the study population (82.17%) were of coloured ethnicity. The remainder were of black ethnicity apart from one child who was of white ethnicity. To determine if there was an increased odds of tuberculosis disease in the coloured population, those of coloured ethnicity were compared to those of other ethnicity.

The proportion of males in this cohort was slightly higher than females, with 50.98% male.

The mean birth weight was 2.78 kg (range: 1.08 – 5.55). Low birth weight was recorded in 27.25% of the study children, which is a higher figure than that estimated by WHO of approximately 15% of children born in sub-Saharan Africa with a birth weight less than 2.5 kg (WHO, 2008).

4.3 Characteristics of the study population at time of TB investigation

At the time of investigation for tuberculosis, the median age of the children was 18.13 months (range: 9.0 – 47.53).

The mean haz was -1.18 (range: -5.05 – 2.44) and 27.45% of the study population had a height for age z score of less than -2.

The HIV status of 481 children was known, 2.91% of the children tested positive for HIV.

182 (35.69%) of the study population had previously been treated for helminth infection. The treatment had been administered at the local health clinic. Reasons for helminth treatment included:

- Mother had seen worms in the child's stool
- The child had complained of abdominal discomfort
- Abdominal discomfort on examination
- Poor appetite or poor weight gain

Table 13: Baseline characteristics in cases and controls

(primary analysis n=510)

(Univariate logistic regression of an association between co-variables and tuberculosis)

Variable	Total (n=510)	Cases (n=328)	Controls (n=182)	OR	95% CI
Age at TB investigation (median & range) (mths)	18.13 (9.0–47.53)	18.02 (9.16–47.53)	18.48 (9.0–47.5)	0.99	0.97 – 1.01
Gender (% male)	50.98	54.88	43.96	1.55	1.08 – 2.23
Ethnicity (% coloured)	82.17	83.91	78.95	1.39	0.87 – 2.23
Birth weight (mean & range) (kg)	2.78 (1.08–5.55)	2.75 (1.08–5.55)	2.83 (1.26–3.96)	0.77	0.55 – 1.09
Low birth weight (% birth weight < 2.5 kg)	27.25	28.66	24.73	1.22	0.81 – 1.85
Mother's age at child's birth (mean & range) (yrs)	25.71 (14.18–43.64)	25.65 (15.04–42.17)	25.83 (14.18–43.46)	0.99	0.97 – 1.03
Gestation (median & range) (weeks)	39 (29–44)	39 (30-44)	39 (29–44)	0.98	0.91 – 1.06
Early birth (% born before 37 weeks)	22.16	23.48	19.78	1.24	0.80 – 1.94
Type of housing (% living in an informal dwelling)	9.41	9.45	9.34	1.01	0.54 – 1.89
Type of community (% living on a farm)	23.53	23.78	23.08	1.04	0.68 – 1.60
Number of occupants (median & range)	5 (1–20)	6 (1-20)	5 (1-13)	1.10	1.02 – 1.18
haz (mean & range)	-1.18 (-5.05–2.44)	-1.35 (-5.05–2.34)	-0.86 (-4.95–2.44)	0.78	0.68 – 0.89
Birth site: (% born Worcester)	52.55	48.48	59.89	1.0	
Birth site: (% born Ceres)	18.24	20.12	14.84	1.68	1.01 – 2.80
Birth site: (% born Robertson)	17.25	19.21	13.74	1.73	1.02 – 2.92
Birth site: (% born Montagu)	11.96	12.20	11.54	1.31	0.73 – 2.34
HIV status (%HIV exposed)	2.91	3.58	1.72	2.12	0.58 – 7.70
Helminth treatment (% treated)	35.69	35.98	35.16	1.04	0.71 – 1.51

IQR – interquartile range

CI – confidence interval

Table 14: Baseline characteristics in cases and controls in the reduced sample
(primary analysis n=435)

(Univariate logistic regression of an association between co-variables and tuberculosis)

Variable	Total (n=435)	Cases (n=280)	Controls (n=155)	OR	95% CI
Age at TB investigation (median & range) (mths)	17.80 (9.0–47.53)	17.55 (9.2-47.53)	18.40 (9.0-47.5)	0.98	0.96 – 1.01
Gender (% male)	51.03	55.36	43.23	1.63	1.10 – 2.42
Ethnicity (% coloured)	82.07	83.57	79.35	1.30	0.80 – 2.18
Birth weight (mean & range) (kg)	2.81 (1.08–5.55)	2.78 (1.08-5.55)	2.87 (1.26-3.96)	0.70	0.48 – 1.03
Low birth weight (% birth weight < 2.5 kg)	24.37	26.43	20.65	1.38	0.86 – 2.21
Mother's age at child's birth (mean & range) (yrs)	25.54 (14.18–43.64)	25.47 (15.04-41.70)	25.65 (14.18-43.64)	0.99	0.97 – 1.03
Gestation (median & range) (weeks)	39 (29–44)	39 (30-44)	39 (29-44)	0.94	0.87 – 1.03
Early birth (% born before 37 weeks)	21.15	23.57	16.77	1.53	0.92 – 2.53
Type of housing (% living in an informal dwelling)	8.97	8.57	9.68	0.88	0.44 – 1.72
Type of community (% living on a farm)	23.91	25.36	21.29	1.26	0.79 – 2.01
Number of occupants (median & range)	5 (1–20)	6 (1-20)	5 (1-13)	1.09	1.01 – 1.18
haz (mean & range)	-1.15 (-5.05–2.44)	-1.33 (-5.05-2.34)	-0.82 (-4.95-2.44)	0.77	0.66 – 0.89
Birth site: (% born Worcester)	53.33	48.57	61.94	1.0	
Birth site: (% born Ceres)	17.93	19.64	14.84	1.69	0.97 – 2.93
Birth site: (% born Robertson)	17.70	20.36	12.90	2.01	1.13 – 3.57
Birth site: (% born Montagu)	11.03	11.43	10.32	1.41	0.73 – 2.72
HIV status (% HIV exposed)	2.53	2.86	1.94	1.50	0.39 – 5.70
Helminth treatment (% treated)	34.94	35.36	34.19	1.05	0.70 – 1.59

IQR – interquartile range

CI – confidence interval

The baseline characteristics of the reduced sample are similar to the baseline characteristics of the full sample used for the primary analysis, suggesting that reducing the sample to include only those children who have a complete set of data for the multivariate analysis will not influence the result, as shown in Tables 13 and 14. Both univariate analyses show that tuberculosis is associated with haz, gender, number of occupants sharing the same dwelling as the child and birth site.

4.4 Primary analysis

4.4.1 Potential confounding variables

All co-variables were tested for potential confounding as described in the methods section. Number of occupants sharing the same dwelling as the child and haz are significantly related with tuberculosis in those not exposed to helminth treatment as shown in Table 15. Age and birth site are significantly related to exposure to helminth treatment as shown in Table 16. None of the variables satisfies both criteria for being a potential confounder.

Table 15: Potential confounders
(primary analysis not exposed to helminth Rx)

Co-variable	Cases (n=181)	Controls (n=102)	OR	95% CI
Age at TB investigation (median) (mths)	15.40	14.75	1.01	0.97 – 1.05
Gender (% male)	52.49	45.10	1.34	0.83 – 2.19
Ethnicity (% coloured)	82.874	80.39	1.18	0.63 – 2.20
Birth weight (mean) (kg)	2.78	2.83	0.85	0.53 – 1.35
Low birth weight (% birth weight < 2.5 kg)	25.41	23.53	1.11	0.63 – 1.95
Mother's age at child's birth (mean)	25.51	25.56	1.00	0.96 – 1.04
Gestation (median) (weeks)	39	39	0.95	0.86 – 1.05
Early birth (% born before 37 weeks)	23.76	17.65	1.45	0.79 – 2.69
Type of housing (% living in an informal dwelling)	9.39	10.78	0.86	0.39 – 1.91
Type of community (% living on a farm)	25.97	23.53	1.14	0.65 – 2.01
Number of occupants (median)	6	5	1.13	1.02 – 1.24
haz (mean)	-1.28	-0.89	0.83	0.70 – 0.99
Birth site: (% born Worcester)	56.35	60.78	1.00	
Birth site: (% born Ceres)	14.36	12.75	1.46	0.74 – 2.88
Birth site: (% born Robertson)	9.39	11.76	1.22	0.58 – 2.54
Birth site: (% born Montagu)	9.52	11.86	0.86	0.39 – 1.92
HIV status (% HIV exposed)	2.76	1.96	1.42	0.27 – 7.46

IQR – interquartile range

CI – confidence interval

Table 16: Potential confounders

(primary analysis association between helminth Rx and the co-variables)

Co-variable	Helminth Rx (n=152)	No helminth Rx (n=283)	OR	95% CI
Age at TB investigation (median) (mths)	22.55	15.37	1.15	1.11 – 1.19
Gender (% male)	53.29	49.82	1.15	0.77 – 1.71
Ethnicity (% coloured)	82.24	81.98	1.02	0.61 – 1.70
Birth weight (mean) (kg)	2.83	2.80	1.12	0.77 – 1.64
Low birth weight (% birth weight < 2.5 kg)	23.68	24.73	0.94	0.60 – 1.50
Mother's age at child's birth (mean)	25.56	25.53	1.00	0.97 – 1.03
Gestation (median) (weeks)	39	39	1.05	0.97 – 1.15
Early birth (% born before 37 weeks)	20.39	21.55	0.93	0.57 – 1.52
Type of housing (% living in an informal dwelling)	7.24	9.89	0.71	0.34 – 1.47
Type of community (% living on a farm)	21.71	25.09	0.84	0.52 – 1.32
Number of occupants (median)	5	6	0.97	0.90 – 1.04
haz (mean)	-1.17	-1.14	0.99	0.86 – 1.13
Birth site: (% born in Worcester)	44.74	57.96	1.00	
Birth site: (% born in Ceres)	17.76	18.02	1.28	0.74 – 2.20
Birth site: (% born in Robertson)	25.00	13.78	2.35	1.39 – 3.99
Birth site: (% born Montagu)	12.50	10.24	1.58	0.83 – 3.01
HIV status (% HIV exposed)	2.63	2.47	1.07	0.31 – 3.07

IQR – interquartile range

CI – confidence interval

4.4.2 Univariate analysis

Table 17: Association between helminth treatment and tuberculosis
Model 1 (primary analysis n=510)

Variable	OR	95% CI
Helminth treatment	1.04	0.71 – 1.51

The univariate unadjusted analysis shows no association between tuberculosis and helminth treatment as shown in Table 17.

4.4.3 Multivariate analysis

The logistic regression analysis was performed on 435 children who had a full set of data for all variables included in this analysis.
Observations that had missing data were excluded in the logistic regression analyses as shown in Tables 11 and 12.

Table 18: Association between helminth treatment and tuberculosis
Model 2 (primary analysis n=435)

Variable	OR	95% CI	Akaike's information criterion (AIC)
Helminth treatment	1.05	0.70 – 1.59	570.55

Model building proceeded by adding other risk factors to the model with helminth treatment, including the variables that proved to have a significant univariate association with tuberculosis disease as identified in Table 13.

The model was increased by adding each of the co-variables separately. The best model was selected using the lowest Akaike's information criterion (AIC) and the likelihood ratio test p value was used to determine if this was a statistically significant improvement.

Table 19: Primary analysis

Model 3 (adding each variable to the model of helminth Rx)

Variable	lrtest p-value	AIC
Age at investigation	0.11	569.94
Gender	0.02	566.69
Ethnicity	0.28	571.36
Birth weight	0.06	569.13
Low birth weight	0.17	570.69
Mother's age	0.78	572.47
Gestation	0.18	570.71
Early birth	0.09	569.70
Housing	0.71	572.41
Type of community	0.33	571.61
Number of occupants	0.03	567.62
haz	<0.001	559.28
HIV status	0.55	572.19
Birth site	0.05	568.59

Adding haz results in the most significant improvement to the model that only contained helminth treatment: AIC 559.28; lrtest p value < 0.001 as shown in Table 19.

Table 20: Primary analysis adjusted for haz

Model 4 (adding each variable to the model of helminth Rx and haz)

Variable	lrtest p-value	AIC
Age at investigation	0.23	559.86
Gender	0.02	556.18
Ethnicity	0.43	560.66
Birth weight	0.85	561.24
Low birth weight	0.88	561.26
Mother's age	0.56	560.94
Gestation	0.41	560.61
Early birth	0.23	559.76
Housing	0.82	561.23
Type of community	0.45	560.72
Number of occupants	0.02	555.79
HIV status	0.77	561.19
Birth site	0.11	559.29

Adding number of occupants results in the most significant improvement to the model that contained helminth treatment and haz: AIC 555.79; lrtest p value = 0.02 as shown in Table 20.

Table 21: Primary analysis adjusted for haz and number of occupants

Model 5 (adding each variable to the model of helminth Rx, haz and number of occupants)

Variable	lrtest p-value	AIC
Age at investigation	0.23	556.31
Gender	0.02	552.55
Ethnicity	0.61	557.54
Birth weight	0.96	557.79
Low birth weight	0.97	557.97
Mother's age	0.61	557.54
Gestation	0.50	557.35
Early birth	0.27	556.60
Housing	0.01	557.78
Type of community	0.92	556.88
HIV status	0.70	557.64
Birth site	0.15	556.42

Adding gender results in the most significant improvement to the model that contains helminth treatment, haz and number of occupants: AIC 552.55; lrtest p value = 0.02 as shown in Table 21.

Table 22: Primary analysis adjusted for haz, number of occupants and gender

Model 6 (adding each variable to the model of helminth treatment, haz, number of occupants and gender)

Variable	lrtest p-value	AIC
Age at investigation	0.23	553.09
Ethnicity	0.67	554.37
Birth weight	0.73	554.44
Low birth weight	0.86	554.52
Mother's age	0.50	554.10
Gestation	0.45	553.98
Early birth	0.29	553.42
Housing	0.99	554.55
Type of community	0.38	553.78
HIV status	0.66	554.35
Birth site	0.10	552.36

Adding birth site is an improvement on the model that contains helminth treatment, haz and number of occupants and gender: AIC 552.36; lrtest p value = 0.10 as shown in Table 22. Although this is not a statistically significant result at the 95% level of confidence it was felt that it should be included in the final model as it had a positive association with tuberculosis in the baseline univariate analysis as shown in Table 13.

There is no further improvement to the model by adding additional variables. The best model to examine a relationship between tuberculosis and helminth treatment is a model that includes helminth treatment, haz, number of occupants living in the same dwelling as the child, gender and birth site as shown in Table 23.

Table 23: Primary analysis final model adjusted for haz, number of occupants, gender and birth site

Model 7 (AIC 552.36)

Variable	OR	95% CI
haz	0.78	0.67 – 0.91
Number of occupants	1.10	1.01 – 1.19
Gender	1.67	1.11 – 2.51
Birth site Worcester	1.0	
Birth site Ceres	1.56	0.88 – 2.77
Birth site Robertson	1.94	1.07 – 3.51
Birth site Montagu	1.38	0.71 – 2.71
Helminth treatment	**0.98**	**0.64 – 1.51**

The validity of the model was examined using a plot of residuals versus the linear component of the model as shown in Appendix A figure 3. Pearson chi-square p value of 0.3331 indicates a reasonably good fit, $p > 0.05$.

After adjusting for the effect of haz, number of occupants living in the same dwelling as the child, gender and birth site, there was still no evidence of an association between helminth treatment and tuberculosis disease.

Although the OR changed from an unadjusted OR of 1.05 to 0.98 this is only a difference of 6.6% and the OR is very close to 1 with a 95% CI that includes 1, indicating that there is no relationship between tuberculosis and helminth treatment.

The observed relative difference of 5% is much smaller than the hypothesized 50% relative effect on which the initial power calculations were done at the design phase of the study. Given the proportion of cases and controls treated

for helminth infection in this analysis of 328 cases of tuberculosis and 182 controls of not TB, the study is only 3% powered to prove this reduced relative difference statistically significant at the 5% level, as shown in Tables 24 and 25.

Table 24: Power of the study
(primary univariate analysis n=510)

Proportion of cases treated for helminth infection	Proportion of controls treated for helminth infection	OR	95% CI	Power
35.98%	35.16%	1.05	0.70 – 1.59	3%

Table 25: Power of the study
(primary multivariate analysis n=435)

Proportion of cases treated for helminth infection	Proportion of controls treated for helminth infection	OR	95% CI	Power
35.36%	34.19%	1.05	0.70 – 1.60	3%

This study showed a much smaller difference in proportion of cases and controls exposed to helminth treatment than used in the original sample size calculations. Thus, the study ended up being under-powered. Table 24 shows that for the univariate analysis of the association between helminth treatment and tuberculosis, there was only 3% power to prove the observed effect as measured by an OR of 1.05 to be statistically significant at the 5% level. This power remains at 3% for the reduced sample used in the multivariate model as shown in Table 25.

Once the best model had been selected using 435 children who had a full set of data for all variables included in this sub-set, as shown in Table 23, the multivariate analysis was repeated using as many observations as possible as shown in Table 26.

Table 26: Primary analysis adjusted for haz, number of occupants, gender and birth site (n=493)

Model 8 (AIC 552.36)

Variable	OR	95% CI
haz	0.79	0.69 – 0.91
Number of occupants	1.11	1.02 – 1.19
Gender	1.56	1.07 – 2.29
Birth site Worcester	1.0	
Birth site Ceres	1.38	0.81 – 2.34
Birth site Robertson	1.68	0.97 – 2.92
Birth site Montagu	1.42	0.77 – 2.64
Helminth treatment	**1.03**	**0.69 – 1.53**

The result is similar to the model that analysed 435 children. There is no relationship between helminth treatment and tuberculosis. However birth site is no longer significant at the 95% level of confidence.

The validity of the model was examined using a plot of residuals versus the linear component of the model as shown in Appendix A figure 4. Pearson chi-square p value of 0.3786 indicates a reasonably good fit, $p > 0.05$.

4.5 Secondary analysis

(Including those classified as “unlikely TB” with the control group).

Baseline characteristics of the secondary analysis are similar to the baseline characteristics of the children included in the primary analysis as shown in Table 26. Both univariate analyses show that tuberculosis is associated with haz, gender and number of occupants sharing the same dwelling as the child. However birth site is not statistically significantly associated with tuberculosis in the secondary analysis as it was in the primary analysis and HIV status now has a statistically significant association with tuberculosis.

Table 27: Baseline characteristics in cases and controls

(secondary analysis n=847)

(Univariate logistic regression of an association between co-variable and tuberculosis n= 847)

Variable	Total (n=847)	Cases (n=328)	Controls (n=519)	OR	95% CI
Age at TB investigation (median & range) (mths)	18.37 (9–47.53)	18.02 (9.17-47.53)	18.9 (9.0-47.50)	0.99	0.97 – 1.01
Gender (% male)	50.89	54.88	45.12	1.2	0.98 – 1.71
Ethnicity (% coloured)	84.03	83.91	84.11	0.98	0.67 – 1.45
Birth weight (mean & range) (kg)	2.79 (1.08–5.55)	2.75 (1.08-5.55)	2.82 (1.26-4.44)	0.79	0.61 – 1.03
Low birth weight (% birth weight < 2.5 kg)	27.27	28.66	26.4	1.12	0.82 – 1.53
Mother's age (mean & range)	25.85 (14.18–46.56)	25.65 (15.04-42.17)	25.98 (14.18-46.56)	0.99	0.97 – 1.01
Gestation (median & range) (weeks)	39 (29–44)	39 (30-44)	39 (29-44)	0.99	0.94 – 1.05
Early birth (% born before 37 weeks)	21.96	23.44	21.00	1.15	0.83 – 1.16
Type of housing (% living in an informal dwelling)	10.39	9.45	10.98	0.85	0.53 – 1.34
Type of community (% living on a farm)	24.32	23.78	24.66	0.95	0.69 – 1.32
Number of occupants (median & range)	5 (1–20)	6 (1-20)	5 (1-14)	1.05	1.00 – 1.12
HAZ (mean & range)	-1.08 (-5.05–5.07)	-1.45 (-5.05-2.34)	-0.94 (-4.95-5.07)	0.80	0.72 – 0.88
Birth site: (% born Worcester)	51.59	48.48	53.56	0.82	0.62 – 1.08
Birth site: (% born Ceres)	19.60	20.12	19.27	1.06	0.75 – 1.49
Birth site: (% born Robertson)	17.24	19.21	15.99	1.25	0.87 – 1.79
Birth site: (% born Montagu)	11.57	12.20	11.18	1.10	0.72 – 1.70
Helminth treatment (% treated)	38.61	35.98	40.27	0.83	0.63 – 1.11
HIV status (% infected)	2.13	3.58	1.22	3.01	1.10 – 8.22

IQR – interquartile range

CI – confidence interval

All co-variables were tested for potential confounding using the method described earlier. Number of occupants sharing the same dwelling as the child and haz are significantly related with tuberculosis in those not exposed to helminth treatment as shown in Table 28. Age and birth site are significantly related to exposure to helminth treatment as shown in Table 29. None of the variables satisfies both criteria for being a potential confounder.

Table 28: Potential confounders
(secondary analysis not exposed to helminth Rx)

Variable	Cases (n=210)	Controls (n=310)	OR	95% CI
Age at TB investigation (median) (mths)	15.72	14.78	1.01	0.98 – 1.04
Gender (% male)	51.90	46.77	1.23	0.87 – 1.74
Ethnicity (% coloured)	84.23	83.45	1.06	0.65 – 1.73
Birth weight (mean) (kg)	2.75	2.81	0.80	0.57 – 1.12
Low birth weight (% birth weight < 2.5 kg)	28.10	27.10	1.05	0.71 – 1.56
Mother's age (mean)	25.68	25.64	1.00	0.97 – 1.03
Gestation (median) (weeks)	39	38	0.99	0.92 – 1.06
Early birth (% born before 37 weeks)	24.29	20.97	1.21	0.80 – 1.84
Type of housing (% living in an informal dwelling)	9.52	11.29	0.83	0.46 – 1.48
Type of community (% living on a farm)	25.71	26.45	0.96	0.65 – 1.43
Number of occupants (median)	6	5	1.08	1.01 – 1.16
haz (mean)	-1.30	-0.93	0.84	0.74 – 0.95
Birth site: (% born Worcester)	55.72	55.51	1.00	
Birth site: (% born Ceres)	20.95	22.26	0.94	0.60 – 1.46
Birth site: (% born Robertson)	13.81	11.29	1.22	0.71 – 2.10
Birth site: (% born Montagu)	9.52	10.97	0.86	0.47 – 1.58
HIV status (% infected)	3.00	0.67	4.56	0.91 – 22.83
haz (mean)	-1.30	-0.93	0.84	0.74 – 0.95

IQR – interquartile range

CI – confidence interval

Table 29: Potential confounders

(secondary analysis association between helminth Rx and the co-variables)

Variable	Helminth Rx (n=327)	No helminth Rx (n=520)	OR	95% CI
Age at TB investigation (median) (months)	22.90	15.36	1.15	1.12 – 1.18
Gender (% male)	54.13	48.85	1.24	0.94 – 1.63
Ethnicity (% coloured)	88.44	83.77	1.05	0.71 – 1.55
Birth weight (mean) (kg)	2.81	2.78	1.08	0.83 – 1.41
Low birth weight (% birth weight < 2.5 kg)	26.91	27.50	0.97	0.71 – 1.32
Mother's age (mean)	26.16	25.66	1.01	0.99 – 1.03
Gestation (median) (weeks)	39	38	1.05	0.99 – 1.11
Early birth (% born before 37 weeks)	21.41	22.31	0.95	0.68 – 1.33
Type of housing (% living in an informal dwelling)	10.09	10.58	0.95	0.60 – 1.50
Type of community (% living on a farm)	21.41	26.15	0.78	0.55 – 1.07
Number of people living in the same dwelling (median)	6	5	0.98	0.92 – 1.03
haz (mean)	-1.09	-1.07	0.99	0.90 – 1.10
Birth site: (% born Worcester)	45.25	55.58	1.00	
Birth site: (% born Ceres)	16.21	21.73	0.92	0.63 – 1.34
Birth site: (% born Robertson)	25.08	12.31	2.50	1.71 – 3.67
Birth site: (% born Montagu)	13.46	10.38	1.59	1.02 – 2.48
HIV status (% infected)	2.98	1.61	1.88	0.72 – 4.92

IQR – interquartile range

CI – confidence interval

4.5.1 Univariate analysis

Table 30: Association between helminth treatment and tuberculosis
Model 9 (secondary analysis n=847)

Variable	OR	95% CI
Helminth treatment	0.83	0.63 – 1.11

The univariate unadjusted analysis shows a 17% relative reduction in the odds of tuberculosis. However, this result is not statistically significant at the 5% level of significance as shown in Table 30.

4.5.2 Multivariate analysis

The logistic regression analysis was performed on 724 children who had a full set of data for all variables included in this sub-set.

Table 31: Association between helminth treatment and tuberculosis
Model 10 (secondary analysis n=724)

Variable	OR	95% CI	AIC
Helminth treatment	0.83	0.61 – 1.14	968.86

Model building proceeded by adding other risk factors to the model with helminth treatment, including variables that proved to have a significant univariate association with tuberculosis disease, including those identified in Table 27.

The model was increased by adding each of the co-variables separately. The best model was selected by using the lowest Akaike's information criterion (AIC) and the lrtest p value was used to determine if this was a statistically significant improvement.

Table 32: secondary analysis

Model 11 (adding each variable to the model of helminth Rx)

Variable	lrtest p-value	AIC
Age at investigation	0.08	967.81
Gender	0.05	967.07
Ethnicity	0.77	970.78
Birth weight	0.16	968.92
Low birth weight	0.67	970.68
Mother's age	0.45	970.28
Gestation	0.50	970.41
Early birth	0.12	968.40
Housing	0.23	969.45
Type of community	0.22	970.64
Number of occupants	0.03	966.32
haz	<0.001	952.85
HIV status	0.15	968.75
Birth site	0.20	970.23

Adding haz results in the most significant improvement to the model that only contained helminth treatment: AIC 952.85; lrtest p value < 0.001 as shown in Table 32.

Table 33: Secondary analysis adjusted for haz

Model 12 (adding each variable to the model of helminth Rx and haz)

Variable	lrtest p-value	AIC
Age at investigation	0.17	952.97
Gender	0.07	951.46
Ethnicity	0.45	954.27
Birth weight	0.51	954.41
Low birth weight	0.23	953.41
Mother's age	0.18	953.05
Gestation	0.93	954.84
Early birth	0.95	953.89
Housing	0.19	953.11
Type of community	0.94	954.84
Number of occupants	0.02	949.27
HIV status	0.26	953.57
Birth site	0.28	955.00

Adding number of occupants results in the most significant improvement to the model that contained helminth treatment and haz: AIC 949.27; lrtest p value = 0.02 as shown in Table 33.

Table 34: Secondary analysis adjusted for haz and number of occupants

Model 13 (adding each variable to the model of helminth Rx, haz and number of occupants)

Variable	lrtest p-value	AIC
Age at investigation	0.17	949.37
Gender	0.07	947.94
Ethnicity	0.99	950.27
Birth weight	0.47	950.74
Low birth weight	0.21	949.71
Mother's age	0.23	949.80
Gestation	0.85	951.23
Early birth	0.40	950.56
Housing	0.33	950.30
Type of community	0.75	951.16
HIV status	0.21	949.69
Birth site	0.33	951.85

Adding gender results in the most significant improvement to the model that contains helminth treatment, haz and number of occupants: AIC 947.94; lrtest p value = 0.07 as shown in Table 34.

Table 35: Primary analysis adjusted for haz, number of occupants and gender

Model 15 (adding each variable to the model of helminth treatment, haz, number of occupants and gender)

Variable	lrtest p-value	AIC
Age at investigation	0.19	948.26
Ethnicity	0.31	948.91
Birth weight	0.65	949.74
Low birth weight	0.30	948.86
Mother's age	0.16	947.99
Gestation	0.89	949.92
Early birth	0.40	949.23
Housing	0.29	948.83
Type of community	0.82	949.89
HIV status	0.22	948.42
Birth site	0.33	950.53

There is no further improvement to the model by adding additional variables. The best model to examine a relationship between tuberculosis and helminth treatment is a model that includes helminth treatment, haz, number of occupants living in the same dwelling as the child and gender as shown in Table 36.

Table 36: Secondary analysis adjusted for haz, number of occupants and gender

Model 16 (AIC 947.94)

Variable	OR	95% CI
haz	0.79	0.71 – 0.88
Number of occupants	1.08	1.01 – 1.44
Gender	1.33	0.98 – 1.81
Helminth treatment	**0.82**	**0.60 – 1.13**

After adjusting for the effect of haz, gender (not significant at the 5% level of significance) and number of occupants living in the same dwelling as the child, the relationship between helminth treatment and tuberculosis disease remains unchanged as shown in Table 36.

The validity of the model was examined using a plot of residuals versus the linear component of the model as shown in Appendix A figure 5. Pearson chi-square p value of 0.4347 indicates a reasonably good fit, $P > 0.05$.

Given the proportion of cases and controls treated for helminth infection in this analysis of 328 cases of tuberculosis and 519 controls of unlikely or not TB, the study is only 22% powered to prove this reduced relative difference significant at the 5% level of significance, in the unadjusted univariate analysis. This power reduces to 19% in the adjusted multivariate analysis as shown in Tables 37 and 38.

Table 37: Power of the study
(secondary analysis n=847)

Proportion of cases treated for helminth infection	Proportion of controls treated for helminth infection	OR	95% CI	Power
35.98	40.27	0.83	0.63 – 1.11	22%

Table 38: Power of the study
(secondary analysis n=724)

Proportion of cases treated for helminth infection	Proportion of controls treated for helminth infection	OR	95% CI	Power
35.36	39.64	0.82	0.60 – 1.13	19%

Once the best model had been selected using the 724 children who had a full set of data for all variables included in this sub-set as shown in Table 36, the multivariate analysis was repeated using as many observations as possible as shown in Table 39.

Table 39: Secondary analysis adjusted for haz, number of occupants and gender (n=822)

Model 17

Variable	OR	95% CI
haz	0.79	0.72 – 0.88
Number of occupants	1.07	1.01 – 1.13
Gender	1.30	0.97 – 1.72
Helminth treatment	**0.85**	**0.63 – 1.14**

The result is similar to the model that analysed 724 children. There is no relationship between helminth treatment and tuberculosis, the relative reduction in tuberculosis odds is 15% but this was not a statistically significant result as shown in Table 39.

5. DISCUSSION

The principal findings of the primary analysis of this study do not demonstrate that children who have been treated for helminth infection and live in a community with a high tuberculosis burden are better protected against tuberculosis disease than those not treated for helminth infection. While the secondary analysis which included those classified as unlikely tuberculosis in the control group showed a decrease in tuberculosis odds of 17% in the univariate analysis and 15% in the adjusted multivariate analysis, this result was not statistically significant at the 95% level of significance.

One of the strengths of this study was that all the children included in the analysis were investigated for tuberculosis in a hospital ward that had been specifically equipped to perform tests used in the diagnosis of tuberculosis. The procedures and tests were performed by study staff who had been trained in protocol and standardised methods. This standardised method of performing the necessary tests would have minimised the diagnostic measurement error.

As this is a case control study, careful attention was paid to the selection of the control groups. In the primary analysis the control group comprised children who were completely asymptomatic. These children were selected for investigation of tuberculosis as they had been exposed to an adult case of tuberculosis. It was felt that although they could have been less healthy than, and therefore not fully representative of, the underlying population of this age group, it was unlikely that this would have changed the results of the study as the OR was very close to 1.

The secondary analysis was performed to establish if the result of the primary analysis held true when those classified as “unlikely TB” were included in the control group, as well as to increase the power of the study.

As both helminth infection and tuberculosis are strongly associated with loss of weight and malnutrition, multivariate analysis which adjusted for these variables was used.

Factors that could have influenced these results include temporality; it was not known when the child was treated for helminth infection. This could have been some months before the tuberculosis investigation and thus the child had the opportunity to become re-infected with helminths. In addition, this study was not equipped to measure helminth infection prevalence directly; the exposure variable that was analysed was only of a history of helminth treatment.

One of the difficulties in trying to establish an association between tuberculosis and helminth infection is the fact that they are both chronic diseases, therefore it cannot be established with certainty whether helminth infection preceded tuberculosis or if chronic tuberculosis might have led to an increased risk of helminth infection. It is possible that the tuberculosis disease process might have started before helminth treatment and might have been undetected.

It is also not known if the helminth treatment had been effective. It is possible that there were children who had been given helminth treatment but were still infected with helminths and thus remained at greater risk of tuberculosis than others effectively treated.

To validate the accuracy of the mother's recall of previous treatment for helminth infection, a 10% sample of CRFs were compared to the child's RTHC where a record of helminth treatment was recorded by staff at the local health facility. No discrepancies in the 10% sample check was found.

It is unlikely that the mother of the child would have accessed helminth treatment from a source other than the local health centre, but it is possible and if she had she might not have been able to recall this previous helminth treatment accurately.

The results of our study differed from other reported research in that two previous case control studies (Tristao-Sa et al. 2002; Elias et al. 2006) found strong associations between helminth infection (as established by examination of stools) and tuberculosis. However, the primary objectives of these studies were to study the prevalence of helminth infection in relation to tuberculosis disease. In neither of these two studies is it known if any participants had previously been treated for helminth infection. It is possible that a larger proportion of the controls had been treated for helminth infection and this could have been a factor that protected them from tuberculosis.

The populations that were previously studied were also different from the population that was investigated in this study. Both previous case control studies were conducted in adults and older children with an age range of 10–80 years. In contrast, the primary objective of this case control study was to determine if children who had been treated for helminth infection had a reduced risk of tuberculosis in the first five years of life. The focus of this research was thus not on the prevalence of helminths but rather the effect of helminth treatment on tuberculosis risk in young children.

In addition to the above findings, this study found an association between tuberculosis and malnutrition, gender and birth site.

A haz below -2 describes stunting which implies long term malnutrition and poor health (WHO, 2007). A total of 27.25% of the study population presented with a haz less than -2, indicating a high proportion of chronically malnourished children. There was a large and statistically significant difference in the proportion of children with a haz <-2 between the cases and controls, 33.21% and 13.55% respectively (OR 3.17; 95% CI 1.88 – 5.35). Children who present with a haz of <-2 are three times more likely to develop tuberculosis than children who present with a haz of >-2 indicating a strong relationship between tuberculosis and malnutrition.

The association between malnutrition and tuberculosis has been explored by many researchers and these results support previous findings (Elias et al. 2001; Campbell et al. 2006; Alderman et al. 2006).

This analysis also showed that children born at the Ceres Hospital or Robertson Hospital were more likely to be diagnosed with tuberculosis than those born in Worcester at either the Eden Donges Hospital or Maria Pieterse Maternity Health Unit. The reason for this difference is not known.

This study found a statistically significant difference between the odds of tuberculosis in males and females. Male children were 1.5 times more likely to be diagnosed with tuberculosis than female children. Differences in incidence of tuberculosis by gender have been noted in previous adult and adolescent research, with results indicating that males are more at risk of tuberculosis than females (Holmes et al. 1998; Nissapatorn et al. 2006). However this difference has not been noted in children. In a review of gender differences in the epidemiology of tuberculosis, Holmes et al. (1998) found that differences in incidence between genders became apparent only after adolescence. They concluded that this gender differential after adolescence was probably due to under reporting of female cases of tuberculosis in developing regions.

Data reported from the National Tuberculosis Centre in Malaysia from January, 2001, to December, 2002, found that 68% of the tuberculosis cases reported were male. This report suggested that these differences in gender were probably due to increasing patterns of non-adherence to tuberculosis treatment in males and poor socio-economic conditions (Nissapatom et al. 2006).

These gender differences in adults with tuberculosis are confirmed in a report by the WHO (2002); However, they state that in most settings the higher incidence of tuberculosis in males only begins to appear between the ages of 10 and 16 years.

6. CONCLUSION

The benefits of deworming young children have been well documented: reducing the number of young children infected with helminths will result in improved physical growth and cognition as well as in improved development of children (Alderman et al. 2006).

The South African Department of Health policy and guidelines for regular treatment of school-going children for soil transmitted helminth infection state that regular deworming of children aged two to five years is included in the Integrated Management of Childhood Illness (IMCI) case management guidelines and that every child between the ages of one and five years should receive regular six-monthly deworming.

Although this study did not show any effect of helminth treatment on the risk of tuberculosis in young children, the benefit of deworming on weight gain is sufficient justification for regular deworming.

The primary analysis of this observational study does not support the hypothesis that helminth treatment reduces the risk of tuberculosis disease in young children in a high-risk tuberculosis population. None of the variables met a priori for potential confounding. The result was thus unchanged when adjusted for the effect of haz, number of occupants sharing the same dwelling as the child, gender and birth site.

Although the secondary analysis showed a 17% reduction in tuberculosis odds, this was not a statistically significant result. The result was also essentially unchanged when adjusted for the effect of haz, number of occupants sharing the same dwelling as the child and gender.

7. REFERENCES

Adams VJ, Markus MB, Adams JF, Jordaan E, Curtis B, Dhansay MA, Obihara CC, Fincham JE. 2005. Paradoxical helminthiasis and giadiasis in Cape Town, South Africa: Epidemiology and control. *African Health Sciences,* 5(3): 276-280.

Alderman H, Konde-Lule J, Sebuliba I, Bundy D, Hall A. 2006 Effect on weight gain of routinely giving albendazole to preschool children during child health days in Uganda: cluster randomised controlled trial. *British Medical Journal*, 333(7559): 122-124.

Bentwich Z, Kalinkovich A, Weisman Z, Borkow G, Beyers N, Beyers A. 1999. Can eradication of helminthic infections change the face of AIDS and tuberculosis? *Immunology Today,* 20(11): 485-487.

Bergman N. 1995. A 'treatment score' for primary and pulmonary tuberculosis. *The Central African Journal of Medicine*, 41: 1-6.

Borkow G, Leng Q, Weisman Z, Stein M, Galai N, Kalinkovich A, Bentwich Z. 2000. Chronic immune activation associated with intestinal helminth infection results in impaired signal transduction and anergy. *The Journal of Clinical Investigation,* 106(8): 1053-1060.

Brown M, Mawa P, Kaleebu P, Elliot A. 2006. Helminths and HIV infection: epidemiological observations on immunological hypotheses. *Parasite immunology.* [Online]. Available: http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pubmed&pubmedid=17042933 [01 July 2009]

Bulletin of the World Health Organisation. 1997. 75: 11-18.

Bundy D, Sher A, Micheal E. 2000. Good Worms or Bad Worms: Do Worm Infestations Affect the Epidemiological Patterns of Other Diseases? *Parasitology Today,* 16(7): 273-274.

Campbell I, Bah-Sow O. 2006. Pulmonary tuberculosis: diagnosis and treatment. *British Medical Journal.* [Online]. Available: http://bmj.com/cgi/content/full/332/7551/1194 [20 May 2007]

Editorial Anon. The Lancet. Thinking beyond deworming. 2004, *The Lancet*. 364(9450): 1993-1994

Elias D, Wolday D, Akuffo H, Petros B, Bronners U, Britton S. 2001. Effect of deworming on human T cell responses to mycobacterial antigens in helminth-exposed individuals before and after bacille Calmette-Guérin (BCG) vaccination. *Clinical and experimental Immunology*, 123: 219-225.

Elias D, Mengistu G, Akuffo H, Britton S. 2006. Are intestinal helminths risk factors for developing active tuberculosis? *Tropical Medicine and International Health,* 11(4): 551-558.

Elliot A, Kizza M, Quigley M, Ndibazza J, Nampijja M, Muhangi L, Morison L, Namujja P, Muwanga M, Kabatereine N, Whitworth A. 2007. The impact of helminths on the response to immunisation and on the incidence of infection and disease in childhood in Uganda: design of a randomised, double-blind, placebo-controlled, factorial trial of deworming interventions delivered in pregnancy and early childhood. *Clinical Trials,* 4: 42-57.

Fincham J. Helminths, HIV/AIDS and tuberculosis. 2001. *Science in Africa.* [Online]. Available: http://www.scienceinafrica.co.za/2001/december/worms.htm. [23 February 2008]

Grosset J. 2003. Mycobacterium tuberculosis in the Extracellular Compartment: an Underestimated Adversary. *Antimicrobial Agents and Chemotherapy,* 47(3): 833-836.

Hanekom W, Abel B, Scribe J. 2007. Immunology protection against tuberculosis. *South African Medical Journal,* 97(10): 793-977.

Hawkridge A, Hatherill M, Little F, Goetz MA, Barker L, Mahommed H, Sadoff J, Hanekom W, Geiter L, Hussey G. Efficacy of percutaneous versus intradermal BCG in the prevention of tuberculosis in South African infants: randomised trial. 2008. *British Medical journal*. [Online].
Available: http://www.bmj.com/cgi/content/full/337/nov13_1/a2052. [26 February 2009]

Holmes C, Hausler H, Nunn P. A review of sex differences in the epidemiology of tuberculosis. 1998. *International Journal of Tuberculosis and Lung Disease*, 2(2): 96-104.

Lloyd M, Lederman S. 2002. Anthropometry and moderate malnutrition in preschool children. *Indian Journal of Paediatrics,* 69(10): 771-774.

Marais B, Gie R, Hesseling A, Schaaf S, Lombard CI, Enarson D, Beyers N. A Refined Symptom-Based Approach to Diagnose Pulmonary Tuberculosis in Children. 2006. *Pediatrics.* [Online]. Available: http://pediatrics.aappublications.org/cgi/content/full/118/5/e1350. [24 March 2008]

Nicol M, Davies MA, Wood K, Hatherill M, Workman L, Hawkridge T, Hanslo M, Eley B, Wilkinson R, Hanekom W, Beatty D, Hussey G. 2008. A comparison of T-Spot.*TB* and tuberculin skin test for the evaluation of young children at high risk for tuberculosis in a community setting. 2009. *Pediatrics.* [Online].

Available: http://pediatrics.aappublications.org/cgi/content/abstract/123/1/38. [26 February 2009]

Nissapatorn V, Kuppusamy I, Sim B, Kia Fatt Q, Khairul Anuar A. Pulmonary tuberculosis in a hospital setting: Gender differences. 2006. *Journal of the Royal Institute of Public Health*. 120(5): 441-443.

Rook A, Dheda K, Zumla A. 2006. Immune systems in developed and developing countries; implications for the design of vaccines that will work where BCG does not. *Tuberculosis.* [Online]. Available: http://inti.elsevierhealth.com/journals/tube [26 March 2008]

Schaaf S, Marais B, Whitelaw A, Hesseling A, Eley B, Hussey G, Donald P. 2007. Culture-confirmed childhood tuberculosis in Cape Town, South Africa: a review of 596 cases. *BMC Infectious Diseases*. [Online]. Available: http://www.biomedcentral.com/1471/7/140 [26 May 2008]

Smuts CM, Faber M, Fincham JE, Oelofse A, Benade AJS. 2004. Integrated Nutritional Programme (INP). Baseline Survey in KwaZulu-Natal and the Eastern Cape. Part 1. Medical Research and Health Systems Trust collaborative study. Community based survey. South African Medical Research Council, Cape Town.

South African National Department of Health. Regular treatment of school-going children for soil-transmitted helminth infections and bilharzia: Policy and implementation guidelines. [Online]. Available http://www.doh.gov.za/docs/factsheets/guidelines/bilhazia/treatment.pdf/ [11-February 2009]

Tristao-Sa R, Ribeiro-Rodrigues R, Johnson L, Pereira F, Dietze R. 2002. Intestinal nematodes and pulmonary tuberculosis: *Revista de Sociedade Brasileira de Medicina Tropical,* 35(5)533: 535.

Walls T, Shingadia D. 2004. Global epidemiology of paediatric tuberculosis. *Journal of Infection.* [Online]. Available: http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6WJT-4979HRF-4&_user=635696&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000033878&_version=1&_urlVersion=0&_userid=635696&md5=b4579ae2aeb7e46072fc31f37b9fd55b [24 May 2008]

Waterlow JC. 1972. Classification and definition of protein-calorie malnutrition. *BMJ*, 3(5826): 566-569.

WHO. Glossary of Terms. [Online]. Available: http://www.who.int/reproductive-health/publications/interagency_manual_on_RH_in_refugee_situations/a3.pdf [13 February 2008]

WHO. 2002. Gender and Tuberculosis.[Online]. Available. http://www.who.int/gender/documents/en/TB.factsheet.pdf [03 August 2009]

WHO. 2005. Health and the Millennium Development Goals. [Online]. Available: http://www.who.int/mdg/publications/mdg_report/MDG_FAST_FACTS.pdf [13 February 2008]

WHO. 2007. Standard deviation of anthropometric Z-scores as a data quality assessment tool using the 2006 WHO growth standards: a cross country analysis. [Online]. Available: http://www.who.int/bulletin/volumes/85/6/06-034421/en/ [08 February 2009]

WHO. 2008. Low birth weight. Country, regional and global estimates. [Online]. Available: http://www.who.int/reproductive-health/publications/low_birthweight/ [08 February 2009]

APPENDIX A: MODEL CHECKING GRAPHS

Figure 3: Plot of residuals versus the linear component of the model
(Primary analysis of 435 children)

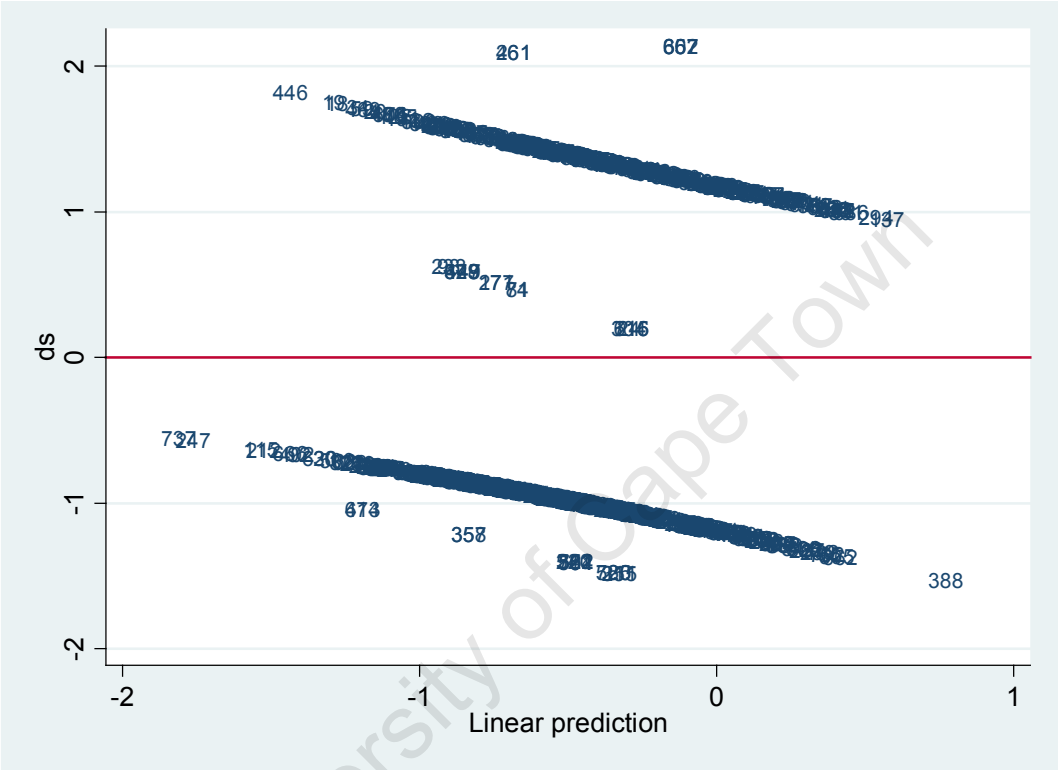


Figure 4: Plot of residuals versus the linear component of the model.
(Primary analysis of 493 children)

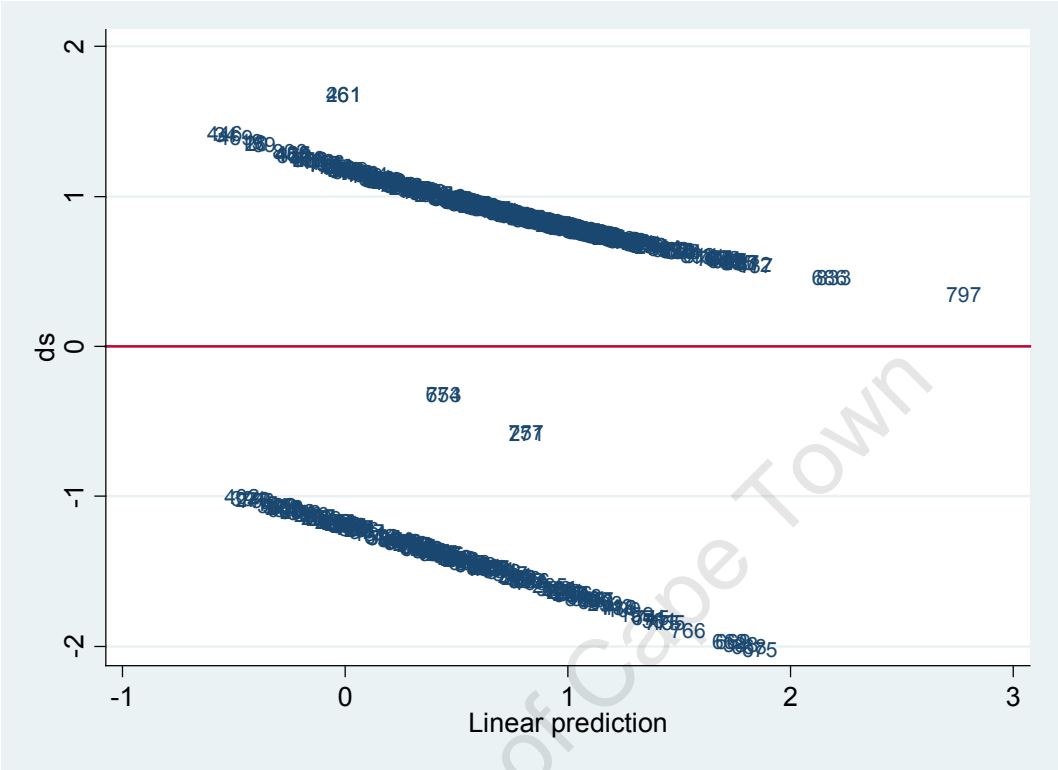


Figure 5: Plot of residuals versus the linear component of the model.
(Secondary analysis of 724 children)